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# Extracellular ATP signaling in plants

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## Abstract

Extracellular adenosine-5′-triphosphate (ATP) induces a number of cellular responses in plants and animals. Some of the molecular components for purinergic signaling in animal cells appear to be lacking in plant cells, although some cellular responses are similar in both systems [e.g. increased levels of cytosolic free calcium, nitric oxide (NO), and reactive oxygen species (ROS)]. The purpose of this review is to compare and contrast purinergic signaling mechanisms in animal and plant cells. This comparison will aid our overall understanding of plant physiology and also provide details of the general fundamentals of extracellular ATP signaling in eukaryotes.

## Extracellular ATP in plants

In the 1970's the essential energy currency molecule of the cell, ATP, was hypothesized to be released into the extracellular milieu and to function as a signaling compound for animal cells [1]. Scientific interest in extracellular ATP accelerated after the first purinoceptor was cloned and characterized from brain tissue in the early 1990's [2]. Extracellular ATP and other nucleotides are now widely accepted as signaling molecules mediating numerous animal cellular processes, including neurotransmission, immune responses, cell growth, and cell death [3].

General acceptance that extracellular ATP is a signaling molecule in plants, however, is relatively recent. In hindsight, clues to a possible role in plants of extracellular ATP have been around since the 1970's when exogenously applied ATP was found to stimulate closure of the venus flytrap [4], endonuclease activity in excised oat leaves [5], and potassium ion uptake into the cells of maize leaf slices [6]. More recent studies demonstrated that extracellular ATP is involved in diverse physiological processes in plant growth and development, including root-hair growth [7,8], pollen-tube growth [9], vegetative growth [10], biotic/abiotic stress responses [11–14], gravitropism [15], cell viability [16], and thigmotropism [17].

Further evidence for a fundamental role of extracellular ATP in the regulation of plant growth and development comes from studies of ecto-apyrases. These enzymes have a

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predicted extracellular catalytic domain [18,19] and hydrolyze nucleoside tri- and di-phosphates to nucleoside mono-phosphate. Therefore they probably act by hydrolyzing extracellular ATP in the apoplast (the free diffusional space outside the plasma membrane; typically limited to the cell wall and intercellular spaces), perhaps to finely regulate extracellular ATP concentration. Double-knockout mutants of the *Arabidopsis* ecto-apyrase genes, in which endogenous extracellular ATP should accumulate excessively, confer male sterility due to abortion of pollen germination [20]. Interestingly, complemented lines for the male sterile phenotype proved to be seedling lethal, exhibiting an extremely malformed dwarf phenotype due to the lack of a functional shoot and root meristem [10,21]. These data are reminiscent of the effects of manipulating the levels of mammalian CD39 ecto-apyrase which functions in regulating inflammation, immune responsiveness, and cell death [22]. As in animal cells, plant extracellular ATP also plays an important role in cell viability, because artificial removal of ATP from the plant apoplast triggers cell death in both cell cultures and whole plants [16]. These data provide strong but indirect evidence for extracellular ATP function in plants and suggest a more fundamental role for extracellular ATP in the maintenance of cell viability.

## ATP in the extracellular matrix of the plant cell

The presence of extracellular ATP presupposes a mechanism by which this highly charged molecule is released into the apoplast. A simple mechanism for ATP release is cell lysis through wounding that affords a passive route of ATP release. Song *et al.* [13] measured ATP levels up to 40 μM in cellular fluid extracted from *Arabidopsis* wound sites (Table 1). They showed that extracellular ATP triggers the formation of ROS, and the expression of several stress-related genes. Therefore, extracellular ATP could be an important signaling molecule for damage by herbivore-and pathogen-induced cell lysis. Such a role is reminiscent of animal cells where released ATP plays an important role in the inflammatory response [23].

Extracellular ATP might also arise by direct secretion (Table 1). This directed release of ATP appears to function during the response to various stimuli, including pathogen elicitors (chitin and yeast extract elicitors [7,14]), abiotic stress (hypertonic stress [12,24]), and touch stimuli [12,17]. The release of ATP from root cells was directly imaged using a luciferase construct engineered to bind to plant cell-wall cellulose, and this demonstrated that the highest areas of extracellular ATP concentration corresponded to those parts of the roots undergoing the most active cellular expansion [7]. Treatment of roots with a calcium chelator and brefeldin A, an inhibitor of vesicular transport, blocked ATP release, suggesting that extracellular ATP was released by vesicular exocytosis in areas of plant growth by a calcium-dependent mechanism. Recently, using this biosensor, release of ATP was measured from root cells in response to touch [17].

By analogy with animals, the mechanism of plant purinergic signaling should include systems for cellular release and removal of the compound from the extracellular space. Inmammalian cells, release of ATP and other nucleotides is mediated by anion channels, gap-junction hemichannels, ATP-binding cassette (ABC) transporters, and exocytosis [25–27]. The nucleotides are rapidly hydrolyzed by ecto-nucleotidases or ecto-apyrases [28].

Interestingly, CD39 ecto-apyrase can form hetero-oligomer complexes with G protein-coupled receptors for nucleotides or nucleosides [29]. Thus, purinergic signaling is achieved via the expression of receptors and ecto-enzymes and through their direct interaction within a multifarious membrane network. In plants, both transport (via an ABC transporter, PGP1) and exocytosis have been suggested as mechanisms for ATP release [7,30] (Figure 1). Other systems, namely anion channels and hemichannels, have yet to be carefully examined in plants (although it should be noted that plants lack canonical hemichannels).

As discussed above, it is likely that extracellular apyrases modulate extracellular ATP levels by rapid hydrolysis [31,32]. An experimental system using potato tuber slices, which enabled direct access to the apoplast without cytosolic contamination [18], permitted characterization of an adenosine nucleosidase bound to the cell wall, as well as apyrase and 5′-nucleotidase as apoplastic enzymes [33]. These data indicate the presence of an ATP salvage pathway in the apoplast of plant cells. Therefore, although the exact molecular mechanisms probably differ, plant and animal cells release ATP and modulate its extracellular concentration by hydrolysis.

## Plant extracellular ATP induces common cellular responses

Animal purinergic receptors are of two types, P2X ion channels and P2Y G-protein-coupled seven-transmembrane receptors [3]. Extensive efforts to identify plant homologs of these animal receptors by sequence and structural comparison algorithms failed to identify canonical P2 receptors [34,35]. Recently, a candidate P2X receptor was identified by a BLAST (basic local alignment search tool)-based search of the green alga *Ostreococcus tauri* genome [36]. However, a BLAST search using this algal sequence failed to find homologs in higher plants (Tanaka *et al.*, unpublished). Hence, if purinergic receptors exist in plants, they are either unique or highly diverged from their animal and algal counterparts.

However, it is clear that extracellular ATP is recognized by plants and can trigger responses similar to those found in animal cells. For example, using transgenic *Arabidopsis* expressing the $Ca^{2+}$-sensitive luminescent protein, aequorin [37], it was shown that ATP and ADP triggered an increase in cytosolic free $Ca^{2+}$ levels ($[Ca^{2+}]_{cyt}$) [12,38,39]. In roots, plasma membrane $Ca^{2+}$-permeable channels are likely to contribute to the extracellular ATP-induced $[Ca^{2+}]_{cyt}$ elevation [39]. The production of ROS by extracellular ATP was also observed in various plant tissues [7,13,14,39]. In these cases, ROS production requires NADPH oxidases because extracellular ATP-induced ROS production did not occur in the leaves of *Arabidopsis rbohD* or *rbohF* mutant plants [13] or in the roots of *rbohC* mutants [39]. Application of ATP to the *rbohC* mutant also failed to induce transcription of the stress-related MAP kinase gene, *AtMPK3*, suggesting that the NADPH oxidase-produced ROS are important for subsequent ATP-triggered response cascades [39]. ATP also stimulates production of NO [40–42]. Based on pharmacological studies using *Salvia miltiorrhiza* hairy root system, ATP-induced NO signaling was shown to be mediated by calmodulins, providing a possible mechanism coupling ATP-induced changes in $[Ca^{2+}]_{cyt}$ and subsequent NO production [41]. Recently, ATP-induced NO production in tomato culture cells was found to occur downstream of both phospholipase C and diacylglycerol

kinase activation [43]. Thus, the signaling pathways linking ATP perception to cellular response could utilize some of the same components seen in mammalian cells [44,45].

The extracellular ATP-associated trio of molecules ($Ca^{2+}$, ROS, NO) appears to be linked in plant signaling pathways. Using $Ca^{2+}$ channel blockers, $Ca^{2+}$ signaling was shown to be essential for ATP-induced NO and ROS production [41,43]. It was also demonstrated that whereas ATP-induced NO can stimulate ROS production, ATP-induced ROS cannot induce NO synthesis [41]. Similar phenomena were observed when NO production was stimulated by addition of a fungal elicitor [46]. These reports suggest that extracellular ATP recognition leads to an increase in $[Ca^{2+}]_{cyt}$, and this activates production of downstream messengers, NO and ROS. In some cases NO and ROS also can stimulate $Ca^{2+}$ elevation. Interestingly, using patch-clamp analysis of mature root epidermal protoplasts of the *rbohC Arabidopsis* mutant, Demidchik *et al.* [39] concluded that the initial ATP-induced metabotropic $[Ca^{2+}]_{cyt}$ elevation from internal $Ca^{2+}$ stores activates NADPH oxidase to generate ROS, which in turn activates $Ca^{2+}$ influx through a hyperpolarization-activated, ROS-sensitive $Ca^{2+}$-permeable channel conductance at the plasma membrane. The simultaneous and balanced production of $Ca^{2+}$, ROS, and NO in response to environmental stimuli appears to represent a common event in regulating plant growth, development, and pathogen defense responses [47,48].

## Significance of plant extracellular ATP signaling in plant growth and development

It is clear that ATP is released into the extracellular matrix and is involved in many cellular processes vital for plant growth and development. Addressing precisely how extracellular ATP signaling functions in specific plant processes would provide insight into the physiological significance of plant extracellular ATP and a better understanding of its recognition and signaling. Some of the most compelling evidence linking extracellular ATP signaling to specific cellular and physiological responses comes from studies of obstacle avoidance of roots, pollen germination and growth, and plant–microbe interactions.

### Obstacle avoidance of roots

In mammalian cells, ATP release can signal mechanical stimulation. In airways, mechanical stimulation, such as occurs during breathing, leads to changes in airflow, pressure and stretching of the surface layer, which impose stresses on epithelial cells that in turn regulate processes such as mucus clearance. The mechanical signal is converted to a biochemical response cascade through extracellular ATP [49]. In plants too, mechanical stresses appear to be coupled to the release of extracellular ATP. Indeed, extracellular ATP was found to play an important role in a root avoidance response where sensing mechanical stimulation elicited by contacting an object triggers root growth, allowing it to efficiently circumnavigate obstacles (Box 1) [17]. ATP at nM levels was secreted into the surrounding medium when the *Arabidopsis* root tip was locally touched with a force of ~200 mN, which is within the range that root tips experience when growing through soil. Because bulk ATP measured in the growth medium is the result of diffusion away from tissues, ATP levels at the surface of the cell are assumed to be higher. In fact, in mammalian cells, Yegutkin *et al.* [50] measured

pericellular [ATP] on the surface of lymphocytes using a novel intrinsic ATP sensor, and found that levels at the membrane surface were often 1000-fold higher than in the bulk medium. Exogenously applied ATP at high concentration can change the sensitivity of the root tip to the growth-regulating plant hormone auxin and reduce shootward auxin transport [15]. Therefore, it is conceivable that extracellular ATP released upon touch regulates obstacle avoidance by influencing the auxin response, which might itself be mediated by changing $[Ca^{2+}]_{cyt}$. Using a cellulose-binding domain (CBD)–luciferase reporter system [7] the spatial kinetics of ATP release in response to mechanical stimulation was shown to have an asymmetrical distribution. When roots were touched on one side in either the apex or the elongation zone, a transient increase of ATP release was observed on the touched side. Weaker but more prolonged ATP release was observed on the opposite side (Figure 2). This asymmetrical extracellular ATP release might provide a mechanism for the plant to decipher the direction of the touch and modify root growth accordingly.

**Box 1**

### Obstacle avoidance by roots

When a root encounters a barrier to growth, such as a rock or a hardpan layer of soil, it adopts an avoidance response to circumnavigate the obstacle allowing the root system to efficiently explore the soil. Although the ability of roots to circumvent obstacles has been known for over 100 years [77], the molecular mechanisms behind the phenomenon have only recently begun to be unveiled [78,79]. The mechanically-induced avoidance response in roots is composed of forming two bends as the root tip tracks over the barrier. The mechanical signal appears to downregulate the gravitropic response [78,79] allowing the root to grow sideways over the barrier surface. As a result, the root grows parallel to the obstacle (Figure 2).

The asymmetrical distribution of the kinetics and spatial dynamics of touch-induced ATP release were altered in a double mutant of the heterotrimeric G-protein alpha and beta subunits, *gpa1;agb1* [17], implying a role for G-protein-dependent signaling. However, the mechanism of G-protein coupling in plant cells is dramatically different than in animal cells. In root cells, ATP perception by a membrane receptor does not appear to be linked to cytoplasmic changes directly through a G-protein-coupled receptor. Instead, another signal is involved in modulating ATP signaling to the cell, providing a sensitivity rheostat. Habituation is a common response to agonist addition where repeated stimulation leads to a weaker and weaker response until cells become non-responsive. Indeed, repeated touching of wild-type *Arabidopsis* roots led to decreasing ATP release. Desensitization requires the heterotrimeric G-protein complex because this habituation response was not found when the *gpa1;agb1* roots were repeatedly touched. Consistent with this, time-lapse video showed a distinctly different obstacle-avoidance response of the doublemutant compared to wild-type roots [17]. These differences in growth are probably due to differences in the way that mutant and wild-type plants decipher the touch response, mediated, at least in part, by the release and recognition of extracellular ATP. This finding represents a novel twist to the plant purinergic signaling pathway and introduces the possibility of using a genetic model

approach to some of the unsolved questions in ATP release in eukaryotic cells (e.g. an ATP-secretion screen for desensitization components).

## Pollen germination and tube growth

The finding of pollen-lethality in *Arabidopsis* ecto-apyrase mutants suggests a specific role for extracellular ATP in pollen germination and tube growth [9,10,20,21]. The addition of ATP, but not AMP or phosphate, inhibited both pollen germination and tube growth [9,20], both of which are affected by a variety of environmental conditions (e.g. temperature, pH, drought) and internal regulators (e.g. $Ca^{2+}$, calmodulin, phosphoinositides, protein kinases, cyclic AMP, GTPases, and NO) [51]. Extracellular ATP involvement in pollen growth therefore raises the question of how this extracellular signal is integrated with other signaling pathways/mechanisms. Unlike the normal radial expansion of most plant cells, pollen tubes expand from the tip. Other examples of tip growth include root-hair growth in higher plants, nerve growth and axon guidance in animals, and hyphal growth in fungi.

The intersection of extracellular ATP and NO signaling pathways was characterized by a series of pharmacological studies [9].NO signaling appears to use similar mechanisms in plants and animals [52]. For example, NO activates guanylate cyclase which converts GTP to cGMP. This cGMP can then serve as a second messenger that activates specific cellular responses (i.e. inhibition of pollen germination or tube growth). NO-induced cGMP production is inactivated by phosphodiesterases that degrade cGMP to GMP. Phosphodiesterase inhibitors, which enhance cGMP production, synergistically diminish pollen germination with exogenously applied ATP. Other chemicals, such as NO donors and a non-hydrolyzable cGMP analog, have a similar synergistic effect with ATP. By contrast, the guanylate cyclase inhibitor reverses inhibition of pollen germination by ATP addition. These chemicals have similar effects in pollen-tube growth, indicating that extracellular ATP is required for NO-induced responses in pollen germination and growth. This linkage between NO and ATP is yet to be reported for tip-growth phenomena in other organisms.

Wu *et al.* [21] demonstrated that ATP is constitutively released into the bulk growth medium with the concentration increasing six-fold within the first 15 min of pollen tube growth. Similar ATP releases were found during the growth of roots [17] and root hairs [7]. These authors also demonstrated that addition of polyclonal antibodies to *Arabidopsis* ecto-apyrase, as well as plant apyrase inhibitors [53], increased the concentration of bulk ATP in the pollen culture and reduced the growth of pollen tubes. These data suggest that low [ATP] contributes to pollen growth whereas [ATP] above a certain threshold limits growth via NO-dependent signaling. This would be consistent with a previous hypothesis [54] in which extracellular ATP induces plant growth with a typical bell-shaped dose–response curve. Alternatively, extracellular ATP might only function to reduce pollen germination and tube growth rates, thereby controlling the timing of these processes. Additional studies are required to identify what signaling steps (other than changes in NO production) are involved in ATP signaling during the regulation of pollen tube growth.

## Plant–microbe interaction: plant innate immunity

In an environment that is rich in harmful microbes, the survival of higher eukaryotic organisms depends on efficient pathogen-sensing and rapidly mounted defense responses, which are referred to as innate immunity [55]. One of the main challenges for the innate immune system is discriminating between potential pathogens and self. One mechanism by which this is achieved is through the specific recognition of conserved microbe-specific molecules, termed pathogen-associated molecular patterns (PAMPs) [56]. In mammals, ATP released from inflamed, damaged, or metabolically impaired cells represents a danger signal that plays a major role in activating the innate immune system [57]. To distinguish between harmful and non-harmful microorganisms, mammalian cells detect PAMPs to elicit various responses, such as activation of interleukin (IL)-1β gene transcription and accumulation as a pro-cytokine, which is sequestered in the cell in a biologically inactive form[23].However, if the foreign microorganism also causes cell damage, ATP is released as a danger signal, the $P2X_7$ receptor is activated and IL-1β is secreted. Less is known about the role of extracellular ATP in the plant pathogen response. However, ATP was shown to be released upon PAMP treatment in root-hair cells of *Medicago truncatula* [7] and in hairy root culture cells of *S. miltiorrhiza* [14] (Table 1). In both cell types, antagonists of mammalian P2 receptors or ecto-apyrase activity attenuated elicitor-induced ROS production. These studies suggest that plants could have animal-like systems that couple PAMP elicitation and ATP release to the induction of innate immunity.

Song *et al.* [13] proposed a model in which extracellular ATP positively regulates pathogen-induced responses. This was based on the observation that extracellular ATP rapidly induced ROS and transcriptional activation of *Arabidopsis* genes encoding biosynthetic enzymes for the defense-associated hormones jasmonic acid and ethylene. Both hormones can mediate defense responses, and a burst of ROS is crucial for activating defensive signaling [58]. These studies utilized short treatments with exogenous ATP. By contrast, Chivasa *et al.* [11] showed that prolonged extracellular ATP accumulation had a negative effect on the pathogen response. They demonstrated that extracellular ATP accumulation for more than two days suppressed production of another defense-associated hormone, salicylic acid (SA), and attenuated SA- and tobacco mosaic virus (TMV)-induced protein expression of pathogenesis-related (PR) genes. Moreover, prolonged extracellular ATP depletion induced PR gene expression and enhanced resistance to TMV and *Pseudomonas syringae*. These data suggest that a long-term change in extracellular ATP levels is a negative regulator of plant pathogen-defense pathways and, therefore, extracellular ATP concentrations must be tightly controlled. Proteomics data show that when extracellular ATP is chronically depleted for a week, defense genes are induced and other genes (e.g. related to photosynthesis and ATP synthesis) are downregulated [59], suggesting a strategy of withholding photosynthate from invading pathogens, while producing anti-microbial proteins and metabolites.

## Plant–microbe interaction: symbiosis

A growing body of literature points to a fundamental role for extracellular ATP during the initial signaling events between rhizobia and their legume hosts that lead to the formation of nitrogen-fixing root nodules (Box 2). Legume-specific ecto-apyrases have been analyzed in some detail in *Dolichos biflorus* [60], soybean [61] and *M. truncatula* [62]. In particular, the

soybean ecto-apyrase GS52 was shown to be mildly induced upon rhizobial infection and localized to the plasma membrane [61]. Ectopic expression of GS52 in *Lotus japonicus* resulted in both enhanced infection by rhizobia and higher levels of nodulation [63], whereas RNAi silencing of GS52 markedly reduced nodulation on soybean roots [64]. These results are consistent with earlier studies on soybean and *D. biflorus* showing that treatment of roots with antibodies specific to ecto-apyrases inhibited nodule formation [60,61]. The stimulation of infection by ectopic expression of GS52 in *L. japonicus* is especially interesting because it points to a role for the ecto-apyrase very early in the symbiotic interaction. Rhizobia infect legume roots via root hairs in a process that requires mutual signal exchange between symbiont and host and a complex signaling network [65,66]. The key rhizobial signal in this process is Nod factor which, when added to roots, can trigger early nodulation events (Box 2). Because the lipo-chitin Nod signal is chemically similar to fungal-derived chitin elicitors, known to induce ATP release, it is likely that Nod factor also triggers localized extracellular ATP production. This release of extracellular ATP could somehow modulate downstream signaling events crucial for rhizobial infection and nodulation. Release of ROS, NO and elevation of $[Ca^{2+}]_{cyt}$ are all cellular responses known to be induced by extracellular ATP and are among the first cellular responses to rhizobial and/or Nod factor inoculation.

**Box 2**

**A brief overview of the nodulation process**

Nodulation is a highly host-specific interaction in which specific bacterial strains, rhizobia, infect a limited range of plant hosts. Interaction is initiated when plants secrete (iso)flavonoids that are recognized by compatible bacteria, resulting in synthesis of a specific lipo-chitooligosaccharide nodulation signaling molecule (Nod factor) that then activates many of the early events in the root-hair infection process [65]. The infection process occurs when bacteria enter the plant, via the root epidermis, and induce the reprogramming of root cortical cell development, which leads to the formation of a novel organ, the nodule. In the best-studied systems, infection occurs through root hairs which are single, elongated projections from the surface of epidermal root cells. In the nodule, the bacteria convert $N_2$ gas (a non-usable form for plants) to $NH_3$ (which can be used by plants). Because cellular specialization occurs during nodulation, the nodule is a true organ. For example, in addition to infected plant cells, uninfected plant cells also carry out the function of nitrogen assimilation and a well-developed, symplastic transport system allows the exchange of nutrients between the nodule and peripheral vascular tissues [80].

## Recognition mechanism for extracellular ATP in plants

The above discussion clearly demonstrates that plants release ATP and that, once released, ATP can play an important signaling role. Indeed, many of the plant cellular responses are similar to those found in animal systems. However, it remains unclear precisely how ATP is recognized (Figure 3). Given that this nucleotide cannot freely diffuse across the plasma membrane [25,67], exogenously applied ATP probably signals through interaction with membrane-associated receptor proteins. However, as discussed above, extensive sequence-

based surveys failed to identify plant proteins similar to the animal P2X and P2Y purinergic receptors.

It is possible that plants recognize extracellular ATP through a mechanism unrelated to the classical animal plasma membrane receptor model. The plant extracellular matrix contains a suite of secreted proteins capable of being phosphorylated by ATP [68,69]. Therefore, extracellular ATP may modulate the kinome or phosphatome network in the extracellular matrix or cell surface area, which in turn could signal into the cell. Chivasa *et al.* [70] applied exogenous ATP and detected novel phosphorylated ATP-binding proteins with in the extracellular matrix, each of which had a kinase domain. However, Demidchik *et al.* [39] were able to elicit extracellular ATP responses in protoplasts isolated from *Arabidopsis* roots, suggesting that in this case at least, an intact extracellular matrix is not essential for recognition of extracellular ATP. Moreover, the observations that ADP and poorly hydrolyzable analogs (e.g. ATPγS and ADPβS), which are not substrates for kinases or phosphatases, also induce purinergic responses in plants suggest that direct action on protein phosphorylation is unlikely to be a complete explanation. It is also possible that nucleotide-binding annexins could contribute to some of the nucleotide-induced $Ca^{2+}$ conductance changes [71–73].

As with animal extracellular ATP systems, the identification of a plant purinergic receptor or recognition mechanism is of crucial importance to our understanding of extracellular ATP in plants. Although studies clearly show a role for extracellular ATP in various signaling networks and responses to environmental stimuli, a fundamental role for extracellular ATP in plant growth and cell viability is also supported by the current literature. Understanding this particular extracellular ATP function will significantly enhance our knowledge of plant cell physiology. An understanding of the similarities and differences between divergent species also reveals the potential for manipulating purinergic signaling by genetic or therapeutic approaches. The continuing comparison of animal and plant purinergic signaling pathways and responses will ultimately help elucidate the fundamentals of this signaling pathway in eukaryotes and highlight possible plant-specific components of this regulatory system. These studies should also yield crucial clues to discovering so-far unknown purinergic receptors in other organisms, such as yeast, bacteria, and lower animals, each of which is known to respond to extracellular ATP [74–76].

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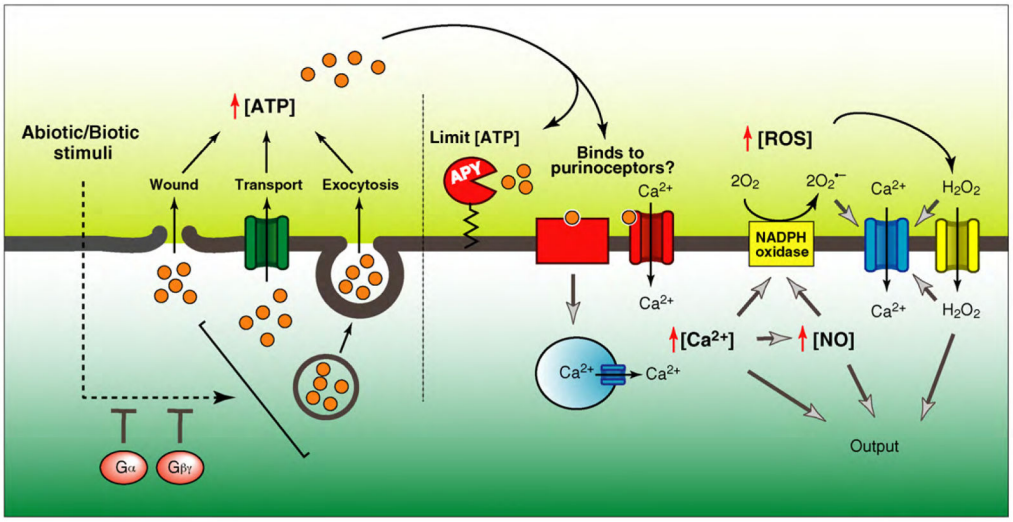


**Figure 1.**

Synopsis of extracellular ATP signaling in plants. In addition to wounding (i.e. cell lysis), a variety of stimuli (e.g. elicitors, touch, hypertonicity, pathogen elicitors, etc.) can induce ATP release via ABC transporter or exocytotic secretion. Heterotrimeric G proteins (Gα and Gβγ complex) appear to be involved in desensitizing stimulus-induced ATP release [17]. The concentration of extracellular ATP is probably further modulated through the action of ecto-apyrases (APY). Elevation of extracellular ATP levels presumably activates an extracellular ATP recognition mechanism (putative purinoceptors), which in turn can elevate cytosolic free $Ca^{2+}$ concentrations. This causes production of NO or ROS through activation of NADPH oxidases. A secondary increase in intracellular $Ca^{2+}$ is the result of activation of $Ca^{2+}$ influx by ROS such as $O_2^{\bullet-}$ and $H_2O_2$. This second messenger trio (NO, ROS, $Ca^{2+}$) activates cellular signaling pathways that lead to induction of a variety of physiological responses, including activation of gene expression (output).

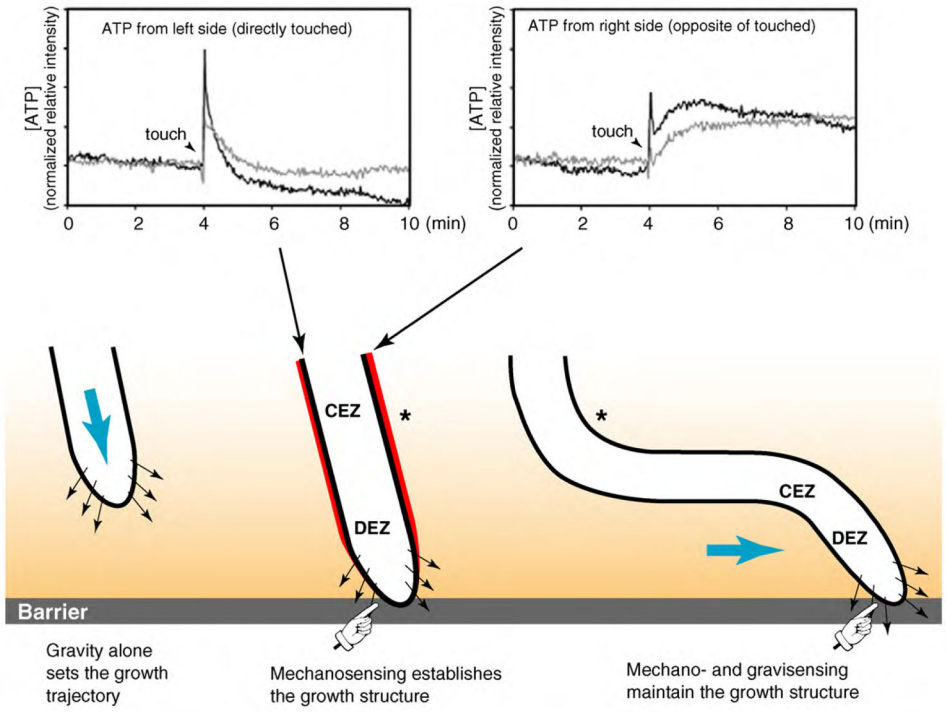


**Figure 2.**

A model for ATP-mediated signaling in touch-sensing during the obstacle-avoidance response in *Arabidopsis* root. Shown at three time points, a root grows to encounter a barrier. The downward trajectory of root growth is driven by the gravity vector. This root constitutively releases ATP primarily at the root tip (small arrows) [17]. Upon encountering a barrier, the root is mechanically stimulated, which causes a transient increase in extracellular ATP level (upper left graph) on one side, and a prolonged increase in extracellular ATP level on the opposite side (upper right graph). ATP could cause a local increase in cytoplasmic $Ca^{2+}$ concentration, and possibly affect local auxin response, which in turn alters the elongation rate in the central elongation zone. Bends in the root then take place through integration of both mechano-and gravity signals. The asterisk marks a fixed reference position on the root when the tip encounters the barrier. CEZ, central elongation zone; DEZ, distal elongation zone. The inset graphs represent ATP changes as indicated by luciferase signal intensity when left side (in the picture) of the tip region (grey trace) or DEZ (black trace) was touched. Data reproduced from Weerasinghe *et al.* [17] with permission from the copyright holder.

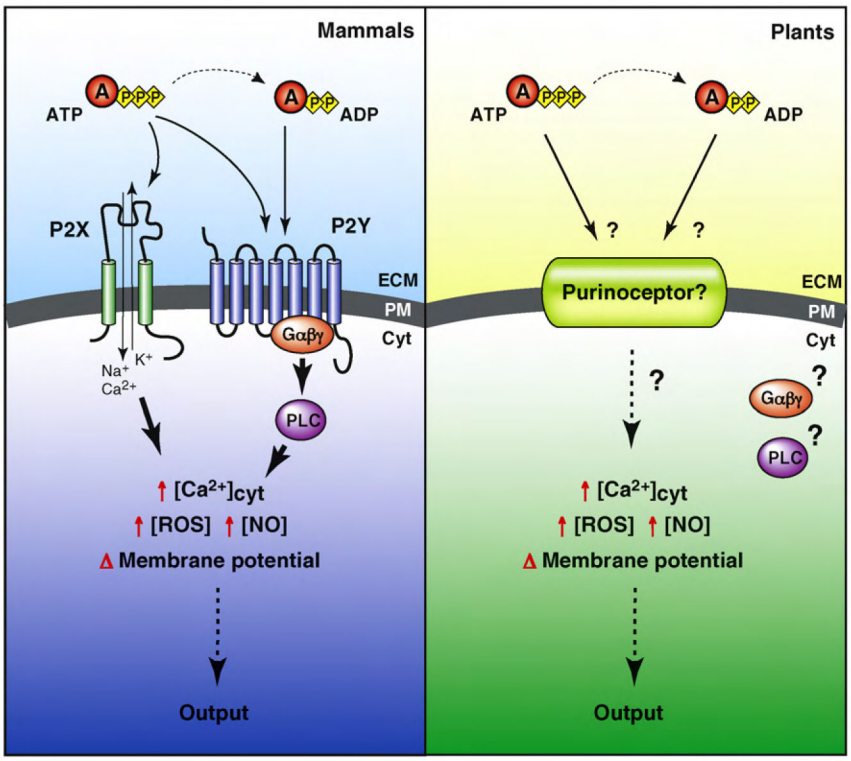


**Figure 3.**

Comparison of the known purinergic signaling pathways of animals to a hypothetical pathway in plants. Several aspects of the cellular responses caused by purinergic stimuli are shared between mammals and plants such as elevation of cytosolic free calcium concentration ($[Ca^{2+}]_{cyt}$), ROS, NO, and membrane conductance changes. In mammals, signaling occurs via P2X ionotropic receptors and P2Y metabotropic receptors via heterotrimeric G protein (Gαβγ) and phospholipase C (PLC) (and occasionally mediated by adenylate cyclase, integrins, and growth factor receptors [83,84]), whereas in plants the purinergic receptor(s) and the exact role of the heterotrimeric G protein have not been identified but are clearly different than in animals. Abbreviations: Cyt, cytoplasm; ECM, extracellular matrix; PM, plasma membrane.

**Table 1**

Release of extracellular ATP in plants

| Category | Stimulus | ATP sensor[a,b] | Plant species | Tissue | Note | Refs |
|---|---|---|---|---|---|---|
| Abiotic stress | Wounding | Luciferase | *Arabidopsis* | Rosette leaf | | [13] |
| | Touch | Luciferase | *Arabidopsis* | Whole seedling | | [12] |
| | | CBD–luciferase | *Arabidopsis* | Root tip | | [17] |
| | Hypertonicity | Luciferase | *Arabidopsis* | Whole seedling | | [12,24] |
| Biotic stress | Chitin elicitor | CBD–luciferase | *M. truncatula* | Root hair | | [7] |
| | YE elicitor | Luciferase | *S. miltiorrhiza* | Hairy root culture cell | Blocked by $La^{3+}$, EGTA | [14] |
| | Mycotoxin BEA | Luciferase | Wheat | Leaf tissue | | [81] |
| Spontaneous | Cell growth | Luciferase | *Arabidopsis* | Suspension culture cell | Inhibited by FB1 | [16] |
| | | Luciferase | *Arabidopsis* | Pollen tube | | [21] |
| | | CBD-luciferase | *M. truncatula* | Root hair | Blocked by $Gd^{3+}$, $La^{3+}$, BAPTA, BFA | [7] |
| | | Luciferase | Cotton | Fiber in ovule | | [82] |

[a] [ATP] in the extracellular medium is directly detected by CBD–luciferase.

[b] [ATP] in the bulk medium is measured by native luciferase.

Abbreviations: BAPTA 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; BEA beauvericin; BFA brefeldin A; EGTA ethylene glycol tetraacetic acid; FB1 fumonisin B1; YE yeast extract.